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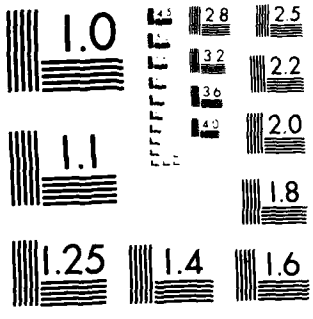

ARE ALL HIGHLY MALIGNANT CANCER CELLS IDENTICAL?

by

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N00014-77-C-0126

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The opinion has been frequently expressed that as cancers become increasingly malignant, they deviate more and more from those of the tissues of their origins and approach a common type, perhaps resembling embryonic tissues (Greenstein, 1947). From comparative studies of cancer cells of different etiology various generalizations have also been made. Thus all cancer cells resemble one another morphologically (Graham, 1972; Knox, 1967). They have similar (and relatively high) lactate production rates (Warburg, 1930), enzyme activities (Greenstein, 1947, 1956), free amino-acid accumulation patterns (Roberts and Tishkoff, 1949; Roberts and Frankel, 1949); and NMR relaxation times of their water protons (Damadian, 1971; Damadian et al, 1973; Ling and Tucker, 1980). All of these generalizations about cancer cells agree with the statement of Greenstein made many years ago: "No matter how or from which tissue tumors arise, they more closely resemble each other chemically than they do normal tissues or than normal tissues resemble each other" (Greenstein, 1954, p. 589; see also 1956).

Another type of findings that have bearings on the subject of carcinogenesis came more recently from the brilliant demonstration of cancer genes, called oncogenes (Weinberg, 1981; Cooper, 1982; Goldfarb et al, 1982; Barbacid (see Reddy et al, 1982). Stimulated by the important studies of tumorigenic retroviruses (see Bishop, 1982, 1983), oncogenes were later detected in a variety of human cancer cells and from cells chemically transformed in vitro. In those cases studied most extensively, the oncogenes were found to differ from its counterpart called protooncogene, believed to be present in all normal cells, by only a single base (see Reddy et al, 1982). How an oncogene and its protein product produce cancer is still not yet fully understood, although there are suggestions that they interfere with cellular differentiation (Beug et al, 1982; Bishop, 1982)

and that although the transformation of protooncogene to oncogene represents a single point mutation, this mutation may represent one of a series of essential steps in a multistage phenomenon comprising carcinogenesis (Weinstein, 1981; Moolgavkar and Knudsen, 1981; Farber and Cameron, 1980).

Following the lead of Greenstein and others, we raised the question, " Is it possible that cancer cells when they reach their ultimate state of autonomy and malignancy become identical in the same sense that identical twins are identical?" To answer this question, we analyzed the total proteins of 15 types of mouse cancer cells of widely different origins, using SDS polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970).

Figure 1 shows the densitometer tracing of SDS-PAGE of the proteins and polypeptides of three normal tissues (liver, lung, and spleen) from four different strains of mice (A: Balb/c; B: $C_3D_2F_i/J$; C: C57BL/6J; D: DBA/2). This figure provides an illustration of the variability among the protein composition of similar organs from the four strains of host mice used for the cancer cells studied as revealed by the technique used. More quantitative assessments of the variability were based on the molecular weight (MW) measurements of the proteins and polypeptides and on the relative abundance of the proteins and polypeptides. The MW's were obtained by comparison of the R_f value with those of eight standards run with each gel (see legend of Fig. 3 for details); the abundance was determined with the densitometer (E-C Scanning Densitometer Model 910) which has a linear absorption response accurate to $1 \pm 1.7\%$ and an integrative device to register density of each band. Thus choosing as an example, in the mouse liver spectrum shown in Fig. 1, we found that for each of the 48 bands observed, the four sets of MW's of each band varied within 1%; i.e., the average standard errors of the mean of all 48 bands as a percentage of the mean MW value was $0.77\% \pm 0.1\%$. The abundance of the four peaks varied more; the standard errors

were 8.8% of the means.

Figure 2 shows the densitometer tracings of the SDS acrylamide gel electrophoretic patterns of the proteins and polypeptides of the hearts and kidneys from representative animals belonging to each of the five vertebrate classes: fish (A); amphibian (B); reptile (C); bird (D); and mammal (E). In each case, the numbered stubs at the bottom of the tracing are molecular weight markers (see Fig. 3 for details). Note that despite the wide evolutionary divergence of the different organ donors, a consistent and distinctive pattern of protein and polypeptide contents is seen for each organ.

Figure 3 presents similar densitometer tracings of the SDS-PAGE of the proteins and polypeptides of the 15 " pure" mouse cancer cells, in the form of washed ascites cells from which red blood cells had been carefully removed by prior treatment with saponin and washing. The 15 sets of tracings were from the following cancer cells: A. T241 (DMBA induced sarcoma); B. Kreb's (carcinoma of the inguinal region); C. Meth. A (fibrosarcoma); D. P4132 (reticulum cell sarcoma); E. P1081 (myelogenous leukemia); F. TA3 (mammary adenocarcinoma); G. Sarcoma 180 (pleomorphic leukocyte sarcoma); H. Reif-Allen (thymoma); I. P815 (mast cell leukemia); J. Ehrlich (mammary adenocarcinoma); K. LSA (lymphoma); L. 15091A (mammary adenocarcinoma); M. L1210 (lymphoid leukemia); N. hepatoma 134 (hepatoma). It is important to point out that all these cancers studied are all highly malignant and autonomous. That is, they are what Potter called " maximally deviated" with transplantation times of about one week (Potter, 1961).

Figure 4, a reproductive of chart D of Fig. 3, demonstrated the band numbering system adopted. Table 1 tabulates the MW's and abundance of a selection of 22 of the more prominent bands. (No significant difference of the other 20 bands of the 42 observable ones were seen.) The MW's vary within the same range as those demonstrated among the proteins and polypeptides of the livers of

four strains of normal mice (i.e., 1%). The abundance shows a variation of 5.8% ± 0.39% for its S.E. of the means and is thus also within the same range as shown for the livers of the normal host mice.

In spite of the widely different origins of these 15 types of cancer cells, the tracings presented in Fig. 3 show remarkable uniformity. There are no greater similarities among the three strains of mouse mammary adenocarcinomas (F, J, L) than between any two cancer cells of the entire collection of tracings shown in Fig. 3.

DISCUSSION

While recognizing that densitometer tracing is not always an accurate way to establish the identity of a specific protein or polypeptide and that each band may represent more than one protein or polypeptide, one can nevertheless state with assurance that the data presented here are in accord with the notion that all 15 mouse cancers of widely different etiology have essentially the same proteins. Polyacrylamide gel electrophoresis reveals that each of the cancer cells studied has the same 42 major bands and no other major bands beyond those 42 bands.

Two conclusions may thus be drawn tentatively from the present findings: (1) when cancer cells have developed to their ultimate state of autonomy and malignancy (i.e., "maximally deviated"), they are either identical or nearly identical; (2) the carcinogenesis process therefore involves a total or partial turning off of those regulatory and structural genes whose activities produce the spectra of proteins specific to each of the normal parent cell types and the turning on of a finite and immutable common set of genes whose activities lead to production of all the proteins which are represented by the 42 bands we discussed, plus others present but are not discerned due to our instrument limitation.

The present findings confirm, extend and round up the series of generaliza-

tions about the nature of cancer cells by Warburg, Greenstein, Roberts, Damadian, and others, if, as we here suggest that all maximally deviated cancer cells are identical. Since all cells from the same living organs carry the same genome, that carcinogenesis could lead eventually to a similar assembly of genes being activated and the suppression of all others is clearly feasible. The sameness of all cancers when " maximally deviated" (if future detailed study can confirm) clearly shows that the genes that determine the cancer cell proteins must be preexisting genes of the cell's normal genome and not new genes resulting from mutations. This view is, of course, in harmony with the repeated demonstration that cancer cells, when transplanted to the proper environments, can develop into normal healthy individuals (Braun, 1953; King and McKinnel, 1960; Mintz, 1978) facts which were in support of the maldifferentiation theory of carcinogenesis (Fischer and Wasels, 1927; Haldane, 1934; Needham, 1950; Pierce, 1967; Markert, 1968; Mintz, 1978).

On the other hand, parodoxically there is also compelling evidence showing that mutation plays a key step in carcinogenesis (Boveri, 1914; Lockhart-Mummery, 1934; Haddow, 1938; Burnet, 1957, 1974): First, almost all carcinogens are mutagens (Mc Cann et al, 1975); secondly, the activation of oncogenes represents a single point mutation. However, the paradox may be only apparent. Thus mutation may be an essential step leading to the eventual expression of the specific genes transcribed in all maximally deviated cancer cells.

That cancer may be related to the embryonic state of the living cell is a familiar conjecture. However, recent years have seen the actual demonstration of a number of proteins seen only in cancer cells and in embryonic tissues but not in adult tissues. Among these "carcinoembryonic" proteins are the α- fetoproteins (Abelev, 1968); carcinoembryonic antigen (Gold and Freedman, 1964); carcinofetal ferritin (Alpert et al, 1973); etc. (for review see Hirai, 1977). Thus it seems not entirely speculation that the maximally deviated cancer cells may in fact represent the

final stage in the reversion to the original embryo cells or even the original fertilized ovum. If this speculation has validity, the carcinogenesis and differentiation have the same destinies but are traversed in opposite directions.

In this light, the fact that protooncogenes are found in such a wide variety of eukaryotic living organs (Bishop, 1983) indicates that it had been carefully conserved over vast genetic distances. If so, one would expect a key role of the protooncogene in the life history of eukaryotic organism. One would then like to ask the question, " Is it possible that the protooncogene in fact represents a master gene whose activity leads to the suppression of the transcription of the genes responsible for the unique set of embryo-cancer proteins and whose mutation releases this suppression?" (A brief abstract of this work was published earlier, Ling and Murphy, 1982).

ACKNOWLEDGMENTS

We are deeply indebted to Dr. Arthur E. Bogden and his associates from the E. G. and G. Mason Research Institute of Worcester, Mass. for providing most of the cancer cell strains used. However, others, no less generous, include Dr. Jose M. Feola of the Department of Radiation Medicine, University of Kentucky; Dr. Isadore Wodinsky of the Arthur D. Little, Inc., Cambridge, Mass; Dr. R. Staroscik, of the Institute for Cancer Research, Fox Chase, Pa.; Drs. G. and T. Gasic, of the Pennsylvania Hospital, Philadelphia, Pa.; and Dr. George Braverman, Tufts University School of Medicine, Boston, Mass. To them we are most thankful.

LEGENDS

Figure 1 - Densitometer Tracings of SDS-PAGE Runs of Normal Liver, Lung and Spleen Tissues from Four Strains of Mice

20-40 mg of normal tissues were frozen in liquid nitrogen and ground in cold stainless tube and grinder. 20 X tissue weight of 50% glycerin was added to the suspension hemogenized. To 0.05 ml of the homogeneous suspension was added 0.2 ml of sample buffer containing 4.0 part 10% SDS, 2.5 part 0.5 M Tris-HCl at pH 6.8, 1.0 part 2 mercaptoethanol, 1.6 part 0.025% Bromphenol Blue, and 6.9 part water. The buffer-tissue mixture was heated in a boiling water bath for 2.5 min. 65 microliters of the cooled sample was placed in each well of the electrophoresis gel. It is absolutely important that for comparison, all samples and standards must be run on the same gel and their densitometer tracings taken at a single setting. The four strains of mice were A. Balb/c; B. $C_3D_2F_i$/J: C. C57BL/6J; D. DBA/2.

Figure 2 - Densitometer Tracings of SDS-PAGE Runs of Normal Heart and Kidney Tissues from Representative Animals of Different Classes

A. Fish (gold fish, Carassius auratus); B. Amphibian (frog, Rana pipiens pipiens); C. Reptile (skink, Scincidae family); D. Bird (chicken, Gallus gallus); and E. Mammals (mouse, ICR). Methods same as described in Figure 1. Numbered stubs correspond to positions of standards from Sigma Chemical Co., St. Louis, MO: 0, lysozyme (MW 14,400 daltons); 1, soybean trypsin inhibitor, 21,500; 2, carbonic anhydrase, 31,000; 3, ovalbumin, 45,000; 4, bovine serum albumin, 66,200; 5, phosphorylase B, 92,500; 6, β-galastosidase, 116,250; and 7, myosin, 200,000.

Figure 3 - Densitometer Tracings of SDS-PAGE Runs of 15 Types of " Pure" Ascites Cancer Cells of Mice

Ascites cells harvested were washed in heparinized mammalian Ringer solution and resuspended with stirring in about 6 to 10 times its volume of Ringer solution containing 0.03% Saponin, centrifuged, washed in Ringer solution, and centrifuged 5 min at 500 g. To the washed cells was added 20 volume of 50% glycerol. To a 0.05 ml aliquot of the homogeneous suspension 0.2 ml sample buffer (see Fig. 1) was added and placed in boiling water bath for 2.5 min. 65 microliters of the cooled solution is placed in each sample well. For the identity of the fifteen strains of cancer cells, see text. Numbered stubs at the bottom refer to position in the gel of different molecular weight standards as indicated. For additional information see Fig. 2.

Figure 4 - The number assignments of the 42 easily recognizable bands of " pure" mouse ascites cancer cells. Densitometer tracing is the same as Fig. 3D.

Table 1 - Molecular weights and abundance of 22 proteins and polypeptides of the fifteen types of " pure" mouse ascites cells shown in Fig. 3. S.E. is the standard error. Abundance is represented as percentage of all the proteins read from the integrated readings of the densitometer.

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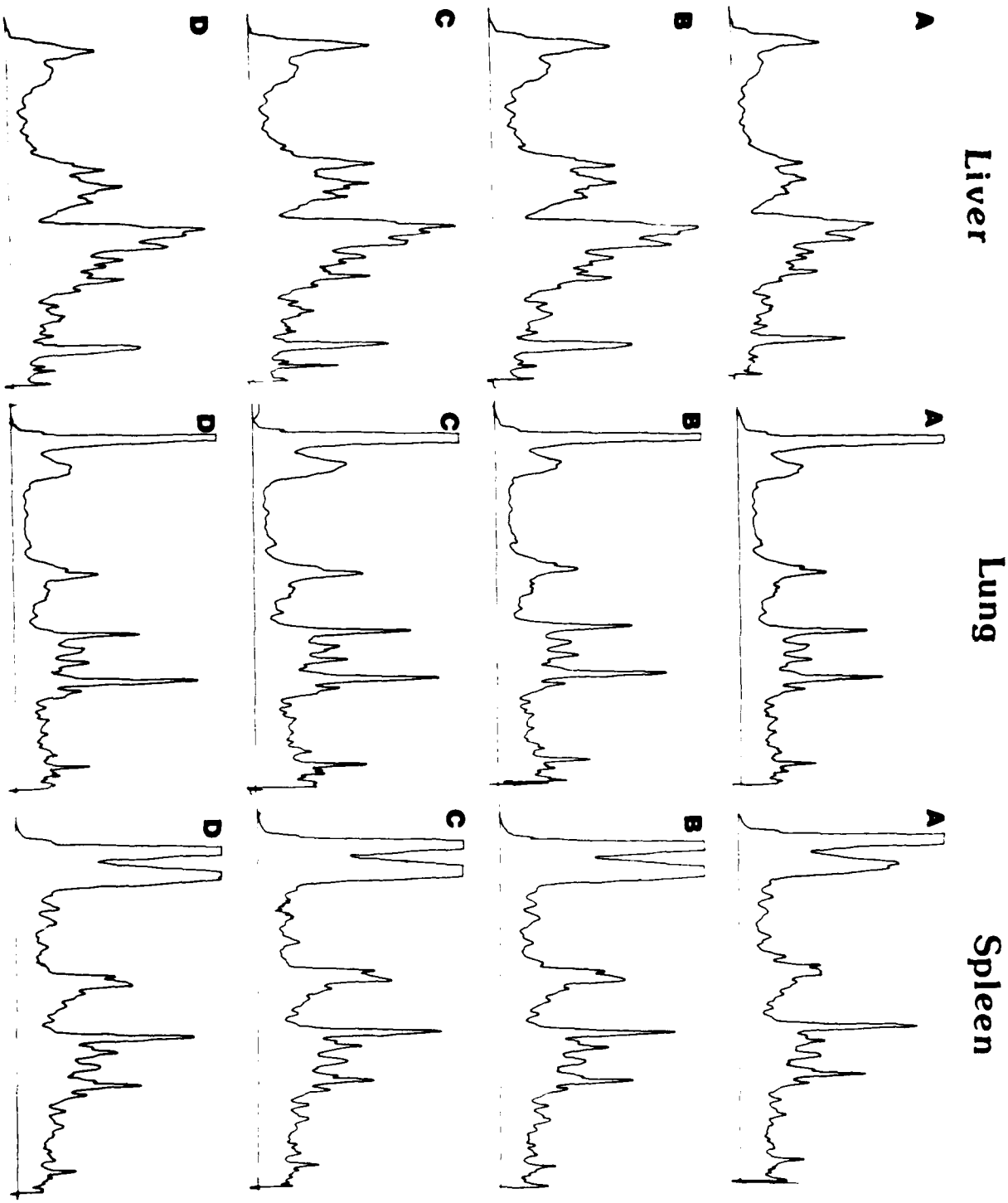
Band Number	Molecular Weights (daltons) Mean±S.E.	S.E./Mean (%)	Abundance (% of total) Mean±S.E.	S.E./Mean (%)
4	202,000±810	0.40	1.52±0.08	5.4
12	92,500±620	0.67	3.22±0.17	5.3
13	83,300±630	0.76	1.67±0.11	6.6
14	77,300±620	0.81	3.04±0.13	4.3
15	69,600±400	0.57	3.93±0.12	3.1
16	64,400±400	0.61	3.62±0.12	3.3
17	59,200±230	0.40	3.76±0.23	6.1
18-19	57,900*±260	0.47	5.6±0.17	3.0
20	53,200±200	0.39	2.07±0.10	4.6
21	51,100±140	0.27	1.98±0.19	9.6
22	49,700±150	0.29	3.79±0.27	7.1
23	48,200±120	0.24	2.10±0.14	6.7
24	46,100±130	1.28	7.16±0.30	4.2
31	36,900±80	0.20	4.19±0.33	7.9
32	35,600±90	0.24	1.95±0.09	4.4
33	34,000±70	0.21	4.79±0.36	7.5
34	32,900±140	0.42	3.12±0.24	7.6
35	31,100±100	0.32	2.19±0.15	6.8
36	29,000±140	0.47	2.53±0.18	7.1
37	26,400±90	0.36	1.52±0.09	5.9
38	25,000±120	0.49	1.27±0.11	8.7
42	16,500±50	0.30	12.70±0.66	5.2
Mean±S.E.		0.41%±0.05%		5.8%±0.39%

*measured at band 18.

TABLE 1



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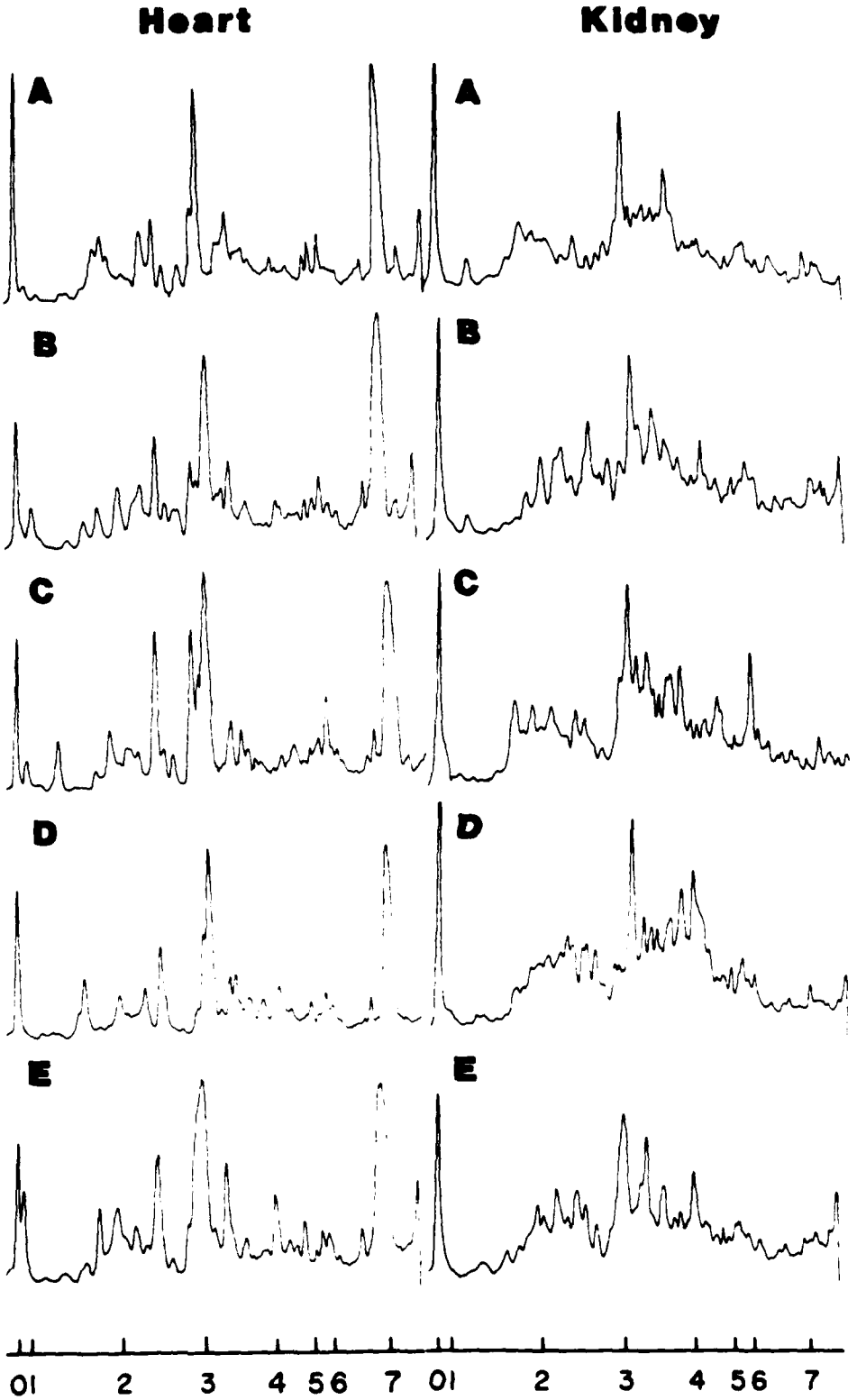

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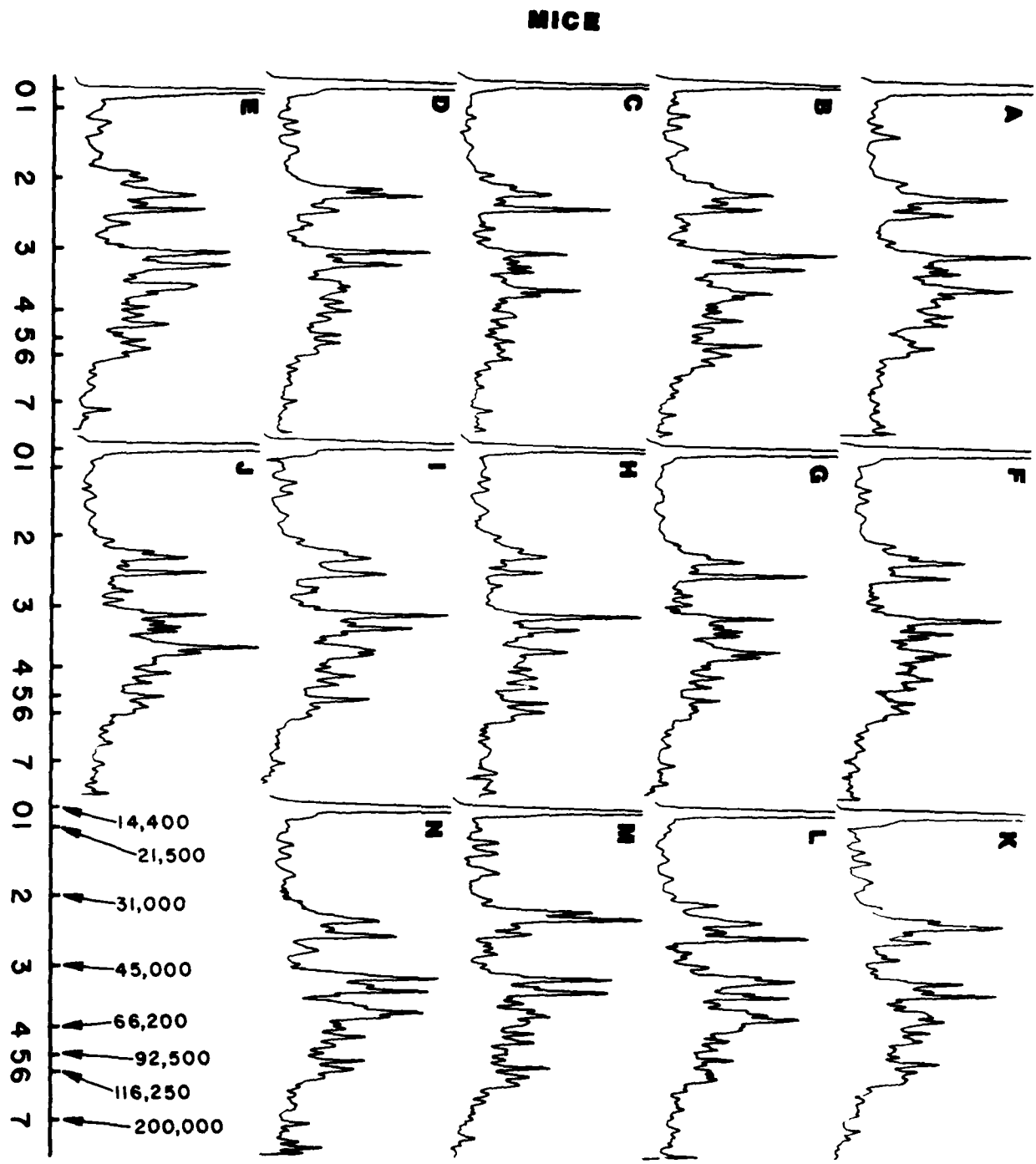
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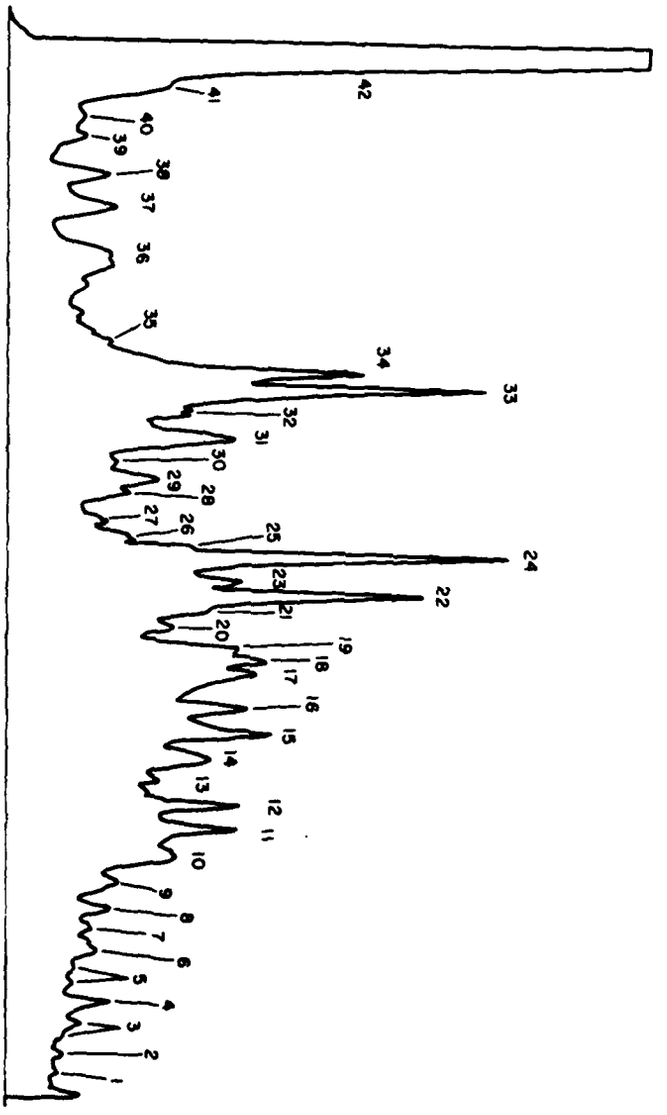
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FIGURE 4